REMARKS

Claims 1-26 were pending, and were rejected by the Examiner. Claims 6, 21-24, and 27-29 have been canceled without prejudice to further prosecution. Applicants have amended claim 1 to incorporate the limitation of claim 6. Thus, amended claim 1 recites a method that includes contacting a tumor cell *in vivo* with a fusion toxin. Claim 12 has been similarly amended to recite a method for killing a glioblastoma tumor cell by contacting it *in vivo* with a fusion toxin. In addition, claim 25 has been amended to recite a pharmaceutical composition containing a fusion toxin, wherein the fusion toxin includes the toxin domain of diphtheria toxin and a urokinase-type plasminogen activator domain. Support for this amendment can be found in previous claim 21. New claims 30-32 depend from claim 25, and recite pharmaceutical compositions containing fusion toxins that correspond to those recited in canceled claims 22-24. Finally, claim 26 has been amended to depend from claim 25. No new matter has been added by these amendments.

In light of these amendments and the following remarks, Applicants respectfully request allowance of claims 1-5, 7-20, 25-26, and 30-32.

Rejection under 35 U.S.C. § 112

The Examiner rejected claim 26 under 35 U.S.C. § 112, second paragraph, as being indefinite. Specifically, the Examiner stated that the term "pharmaceutical composition" lacks proper antecedent basis in claim 21. The Examiner also stated that it is unclear how the limitation "[a]n article of manufacture" would further limit the fusion toxin of claim 21 or the pharmaceutical composition of claim 25.

Applicants have amended claim 26 to refer to the pharmaceutical composition of claim 25. With respect to the Examiner's statement regarding further limitation of claim 25 in claim 26, Applicants submit that claim 26 is a proper dependent claim, as it recites that the pharmaceutical composition of claim 25 is included in an article of manufacture.

In light of the above, Applicants respectfully request withdrawal of the rejection of claim 26 under 35 U.S.C. § 112, second paragraph.

Rejection under 35 U.S.C. § 102

The Examiner rejected claims 12, 13, 15, 17, 18, and 19 under 35 U.S.C. § 102(b) as being anticipated by Rajagopal et al. (*J. Biol. Chem.* 2000, 275:7566-7573). In particular, the Examiner stated that the Rajagopal et al. reference discloses a method for killing a glioblastoma tumor cell, comprising contacting the cell with a fusion toxin that includes (a) the amino terminus of urokinase plasminogen activator, and (b) portions of Pseudomonas exotoxin that include the translocation domain.

Applicants have amended claim 12 to recite a method that includes contacting a glioblastoma tumor cell with a fusion toxin that contains a urokinase-type plasminogen activator domain, wherein the contacting is *in vivo*. Nowhere does the Rajagopal et al. reference teach or suggest a method for killing a glioblastoma tumor cell *in vivo*. Thus, claims 12, 13, 15, and 17-19 are not anticipated by the Rajagopal et al. reference.

In light of the above, Applicants respectfully request withdrawal of the rejection of claims 12, 13, 15, 17, 18, and 19 under 35 U.S.C. § 102(b).

Rejections under 35 U.S.C. § 103

The Examiner rejected claims 12, 13, 15, 17, 18, and 19 under 35 U.S.C. § 103(a) as being unpatentable over Rajagopal et al. (*supra*) in view of Fabbrini et al. (*FASEB J.* 1997, 11:1169-1176), Leppla et al. (U.S. Patent No. 5,591,631), Wels et al. (PCT publication no. WO 94/26308), McDonald et al. (PCT publication no. WO 00/04926), Morishita et al. (*Nucl. Acids Res.* 1996, 35:291-292), Pastan et al. (*J. Biol. Chem.* 1989, 264:15157-15160), Baty et al. (*Mol. Microbiol.* 1988, 2:807-811), el Kouhen et al. (*Eur. J. Biochem.* 1993, 214:635-639), Geoff et al. (*Prot. Eng.* 1997, 10:S5), Bouveret et al. (*Mol. Microbiol.* 1997, 23:909-920), Lacy et al. (*J. Mol. Biol.* 1999, 291:1091-1104), Wiedlocha et al. (*Cancer Res.* 1991, 51:916-920), and Olsnes et al. (*J. Biol. Chem.* 1982, 257:13263-13270). Specifically, the Examiner stated that while the Rajagopal et al. reference does not teach a method of killing a glioblastoma cell by administering a fusion toxin comprising a toxin domain of colicin, anthrax, tetanus, botulinum, saporin, abrin,

bryodin, poke-weed anti-viral protein, viscumin, or gelonin, or an internalization domain of colicin, delta-endotoxin, anthrax, tetanus, or botulinum, the other cited references teach fusion proteins and hybrid toxins comprising toxin domains and/or translocation domains from the listed toxins. Thus, the Examiner alleged that it would have been *prima facie* obvious to one of ordinary skill in the art to substitute another toxin domain for the Pseudomonas exotoxin domain of the fusion toxin taught by Rajagopal et al. The Examiner further alleged that one of ordinary skill in the art would have been motivated to combine the teachings of the cited references with a reasonable expectation of success.

Applicants respectfully disagree. To further prosecution, however, Applicants have amended claim 12 to recite a method for killing a glioblastoma tumor cell *in vivo*, as described above. One of the criteria for establishing a case of *prima facie* obviousness is that all of the claim limitations must be taught or suggested by the prior art. M.P.E.P. § 2143.03. The cited references, either alone or in combination, fail to teach or suggest the *in vivo* methods of amended claims 12, 13, 15, and 17-19. Thus, the references cited by the Examiner do not render these claims *prima facie* obvious.

In light of the above, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

The Examiner rejected claims 1-5 and 7-24 under 35 U.S.C. § 103(a) as being unpatentable over Rajagopal et al. (*supra*) in view of Greenfield et al. (*Science* 1987, 238:536-539) and Fabbrini et al. (*supra*). In particular, the Examiner stated that the Fabbrini et al. reference teaches a method of killing cells that express the urokinase-type plasminogen activator receptor, by contacting the cells with a fusion toxin containing the amino terminal fragment of urokinase-type plasminogen activator and the toxin domain of saporin. The Examiner also stated that the Greenfield et al. reference teaches recombinant forms of diphtheria toxin that retain membrane translocation ability but lose the ability to bind to the diphtheria toxin receptor. Thus, the Examiner alleged that it would have been *prima facie* obvious to one of skill in the art to combine the teachings of Rajagopal et al. with those of Fabbrini et al. and Greenfield et al., and

that the teachings of the cited references would have provided a reasonable expectation of success.

Applicants respectfully disagree. To further prosecution, however, Applicants have amended independent claims 1 and 12 to recite methods that include contacting cells *in vivo* with fusion toxins comprising a urokinase-type plasminogen activator domain. None of the cited references teach or suggest such an *in vivo* method. Since these references fail to teach all of the limitations of claims 1-5 and 7-20, they do not render these claims *prima facie* obvious. The rejection of claims 21-24 is moot, as they have been canceled.

In light of the above, Applicants respectfully request withdrawal of this rejection of claims 1-5 and 7-20 under 35 U.S.C. § 103(a).

Finally, the Examiner rejected claims 1-26 under 35 U.S.C. § 103(a) as being unpatentable over Rajagopal et al. (*supra*) in view of Greenfield et al. (*supra*) and Fabbrini et al. (*supra*), and further in view of Oldfield et al. (*Curr. Topics Microbiol. Immunol.* 1998, 234:97-114) and Mori et al. (*J. Neurooncol.* 2000, 46:115-123). Specifically, the Examiner stated that while neither Rajagopal et al., Greenfield et al., nor Fabbrini et al. teach an *in vivo* method of contacting a tumor cell with a fusion toxin, the Oldfield et al. reference teaches a method of treating a glioblastoma patient with a fusion toxin comprising a mutant diphtheria toxin fused to transferring. The Examiner further stated that the Mori et al. reference teaches that urokinase-type plasminogen activator is unregulated in glioma cells exhibiting enhanced invasion activity. Thus, the Examiner alleged that it would have been *prima facie* obvious to one of ordinary skill to substitute the amino terminal fragment of urokinase-type plasminogen activator for transferrin in the method taught by Oldfield et al. The Examiner also alleged that one of skill in the art would have been motivated to target the plasminogen activator receptor because the cited art teaches that glioblastoma cells would be expected to express numerous such receptor molecules.

Applicants respectfully disagree. Another of the criteria to establish a case of *prima facie* obviousness is that there must have been a reasonable expectation of success for modifying or combining the teachings of the cited references. M.P.E.P. § 2143.02. Applicants submit that the

Examiner has failed to establish a case of *prima facie* obviousness. A person of ordinary skill in the art, reading the cited references at the time the invention was made, would not have had a reasonable expectation of success for using an *in vivo* method to kill tumor cells (e.g., glioblastoma cells) with a fusion toxin comprising portions of the urokinase-type plasminogen activator and diphtheria toxin. The lack of a reasonable expectation of success is due at least in part to the potential for non-target toxicity, which has been well known in the art for a number of years. In fact, the potentially toxic effects of fusion toxins such as DTAT are acknowledged in Applicants' specification. See, for example, page 20, line 12 to page 21, line 6, which discuss the potential for toxicity, and present experimental results showing that *in vivo* use of DTAT did not result in toxicity in the kidneys, liver, spleen, or heart. In particular, the text at page 20, lines 19-21 of the specification stresses the importance of the findings, given that previous immunotoxin studies had resulted in non-target toxicity.

At the time the invention was made, the issue of non-target toxicity was commonly known in the art, and it was well established that anti-vascular immunotoxins had the potential to cause vascular toxicity. Furthermore, a person of ordinary skill in the art would have appreciated that since the brain has a low threshold for bleeding, administration of an anti-vascular fusion toxin to the brain could result in vessel damage, with potential intracranial bleeding and clot formation. This is particularly true in view of the teachings of, for example, Baluna et al. (*Proc. Natl. Acad. Sci. USA* 1999, 96:3957-3962), Hagihara et al. (*Cancer Res.* 2000, 60:230-234), Merrill et al. ("Use of a VEGF-toxin conjugate for treatment of brain tumors," presented at the Keystone Symposium in Salt Lake City, UT in March, 2000), and Hall (*Neurosurg.* 2000, 46:544-551) (copies enclosed).

As disclosed in the Baluna et al. reference, for example, a dose-limiting side effect of fusion toxin therapy is "vascular leak syndrome," which is characterized by an increase in vascular permeability that results in interstitial edema and organ failure. Furthermore, the Hagihara et al. reference discloses that neurological deficits consistent with endothelial damage were observed following interstitial microinfusion convection-enhanced delivery of a transferrin/diphtheria toxin conjugate into brain tumor patients. In fact, the changes that were

observed in the brains of treated patients were consistent with microvascular occlusion and/or petechial hemorrhage. The Merrill et al. reference teaches that intracerebral infusion of a VEGF/diphtheria toxin conjugate resulted in intra- and peritumoral hemorrhages and neurologic deficits. In particular, this reference states, "[T]he usefulness of angiotoxic anti-tumor therapy, in the brain setting particularly, may be complicated by secondary damage to normal surrounding tissue." The Hall reference teaches that at certain doses, intratumoral infusion of a transferrin/diphtheria toxin conjugate resulted in peritumoral brain injury and thrombosis. Thus, a person of ordinary skill in the art at the time the invention was made would not have had a reasonable expectation of success for combining the teachings of the Rajagopal et al., Fabbrini et al., Greenfield et al., Oldfield et al., and Mori et al. references.

Moreover, Applicants submit that a person of ordinary skill in the art at the present time would not have a reasonable expectation of success for combining the teachings of the references cited by the Examiner. More recent publications, such as the Frankel reference (*Clin. Cancer Res.* 2002, 8:942-944) and the Rustamzadeh et al. reference (*J. Neuro-Oncol.* 2003, 65:63-75) (copies enclosed) teach that non-target toxicity of fusion toxins remains a problem to be addressed. For example, the Rustamzadeh et al. reference discloses that because of its anti-vascular effects, DTAT may also promote bleeding, and that future studies will be needed to address the toxicity issues.

In view of the enclosed references and the knowledge in the art, the success of the presently claimed methods (see the specification at page 19, line 18 to page 21, line 6) is surprising and could not have been predicted based on the teachings of the references cited by the Examiner. Thus, the present claims are not *prima facie* obvious. In light of the above, Applicants respectfully request withdrawal of this rejection of claims 1-5, 7-20, and 25-26.

CONCLUSION

Applicants respectfully submit that claims 1-5, 7-20, 25-26, and 30-32 are in condition for allowance, which action is requested. The Examiner is invited to telephone the undersigned if such would further prosecution.

Enclosed is a check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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